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Origin of the dengue virus outbreak in Martin County, Florida, USA 2013

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ABSTRACT

After a 75-year absence from Florida, substantial local transmission of dengue virus (DENV) occurred in Key West, Monroe County, Florida in 2009 and continued in 2010. The outbreak culminated in 85 reported cases. In 2011 and 2012, only isolated cases of local DENV transmission were reported in Florida; none were reported in Key West. In 2013, a new outbreak occurred, but this time in Martin County about 275 miles north of Key West with 22 reported cases. As the Key West and Martin County outbreaks involved DENV serotype 1 (DENV-1), we wanted to investigate whether the same strain or a different strain of DENV was responsible for the outbreaks. In this study, we report the sequence and phylogenetic analysis of the E gene region from a patient diagnosed with dengue in Martin County. Our results indicate that the 2013 Martin County DENV-1 strain is distinct from the 2009–2010 Key West DENV-1 and that it is most closely related to viruses from a recent expansion of South American DENV-1 strains into the Caribbean. We conclude that the 2013 Martin County outbreak was the result of a new introduction of DENV-1 in Florida.

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1. Introduction

Currently, about 40% of the world's population lives in areas at risk of dengue infection (World Health Organization, 2009) and the incidence of dengue is increasing in range and intensity worldwide. A recent meta-analysis estimated 390 million dengue infections per year, more than three times the burden

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previously estimated by the World Health Organization (World Health Organization, 2009; Bhatt et al., 2013). Dengue is caused by a mosquito-transmitted flavivirus, dengue virus (DENV). DENV infection typically manifests as an acute febrile illness with highly variable outcomes ranging from inapparent symptoms to hemorrhagic fever, shock syndrome or even death. There are four distinct serotypes of DENV (DENV-1, -2, -3, and -4). Infection with one serotype typically provides lifetime protection against the same serotype, but the resulting immune response can lead to increased disease severity during a secondary infection with a different serotype. There are currently no commercially available vaccines to prevent DENV infection or specific drugs to inhibit viral replication. The primary means of disease prevention and spread is vector control.

DENV is primarily transmitted by peridomestic *Aedes aegypti* and *Aedes albopictus* mosquito vectors. Both of these species are typically found in tropical and subtropical regions. However, in recent years, the range of these mosquito vectors has increased, leading to a subsequent expansion of the range of DENV transmission (Benedict et al., 2007; Enserink, 2008). Global travel and commerce have played key roles in range expansion and increasing transmission. Transported mosquito adults and larvae, as well as DENV infected travelers returning from regions where DENV is endemic, can initiate de novo local DENV transmission if the mosquito vectors are present. As a result, sporadic outbreaks of locally transmitted DENV have occurred in numerous temperate regions including France, Croatia, and the United States (US) (La Ruche et al., 2010; Schmidt-Chanasit et al., 2010; Centers for Disease Control and Prevention, 2010). According to the US Geological Survey, in 2013, there were 773 laboratory-confirmed imported DENV cases in 41 states (http://diseasemaps.usgs.gov/dep_us_human.html). Given the variable symptoms and lack of clinical experience with DENV in the US, this is almost certainly an underestimate of the true number of imported cases. *A. aegypti* is found in 19 of these states with its range stretching across the southeastern US, up the east coast to New York and west to Kentucky and Indiana, and *A. albopictus* is now established on the Atlantic seaboard from Florida to southern New York (Darsie and Ward, 2005).

In 2009, after a 75-year absence from Florida, a substantial outbreak of locally transmitted DENV occurred in Key West, Monroe County, Florida. According to the Florida Department of Health, twenty-two cases of locally transmitted DENV were confirmed that year (http://www.floridahealth.gov/diseases-and-conditions/mosquito-borne-diseases/surveillance.html). In 2010, an additional sixty-three cases of locally acquired DENV were reported in Monroe County and one case each in Miami-Dade and Broward Counties. The same strain of DENV serotype 1 (DENV-1) was isolated from both mosquitoes and patients in Monroe County, confirming local transmission (Graham et al., 2011; Munoz-Jordan et al., 2013). No further cases of locally acquired DENV have been reported in Monroe County since 2010, suggesting that DENV had been extirpated from the local vector population in that location. However, small numbers of sporadic cases with no travel history have continued in Florida. In 2011, seven additional cases of locally acquired DENV were reported: three cases in Miami-Dade, two in Palm Beach, and one each in Martin and Hillsborough Counties. In 2012, four more cases were reported: two in Miami-Dade and one each in Osceola and Seminole Counties. Most recently, in 2013, another substantial outbreak occurred where twenty-three cases of locally acquired DENV were reported: twenty-two in Martin County and one in Miami-Dade County. The Florida counties with reported cases of locally acquired DENV are shown in Fig. 1.

In this study, we set out to determine whether the locally transmitted DENV strain from Martin County in 2013 is the same as or different from the locally transmitted DENV from Key West in 2009–2010. The answer to this question has major implications for control efforts and epidemiological surveillance. If the two viruses are similar, then that would suggest that a single introduction had spread to multiple areas in Florida due to movement of people and/or mosquitoes within the state. The distance between the neighborhoods of Old Town Key West and Jensen Beach and Rio in Martin County is about 275 miles (Fig. 1), linked much of the way by direct interstate highways. If the two viruses are distinct, that would suggest a new introduction of DENV in Florida from outside the US. Control and surveillance measures to address these two distinct scenarios would differ in focusing on local versus international transport.

Here we report the sequence and phylogenetic analyses of the E protein region of DENV amplified from a single patient diagnosed in Martin County in 2013. Our results indicate that the Martin County and Key West DENV are both DENV-1, but that the two strains are distinct. While the Key West DENV-1 was most closely related to viruses from Nicaragua, the Martin County DENV-1 is most closely related to viruses from a recent expansion of South American DENV-1 viruses into the Caribbean.

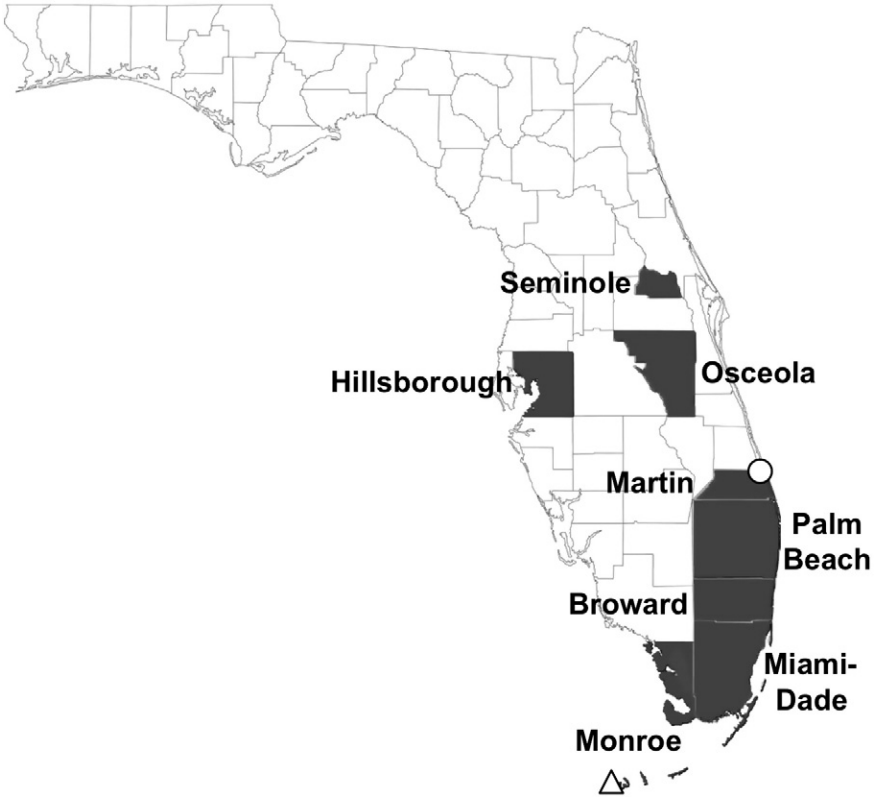
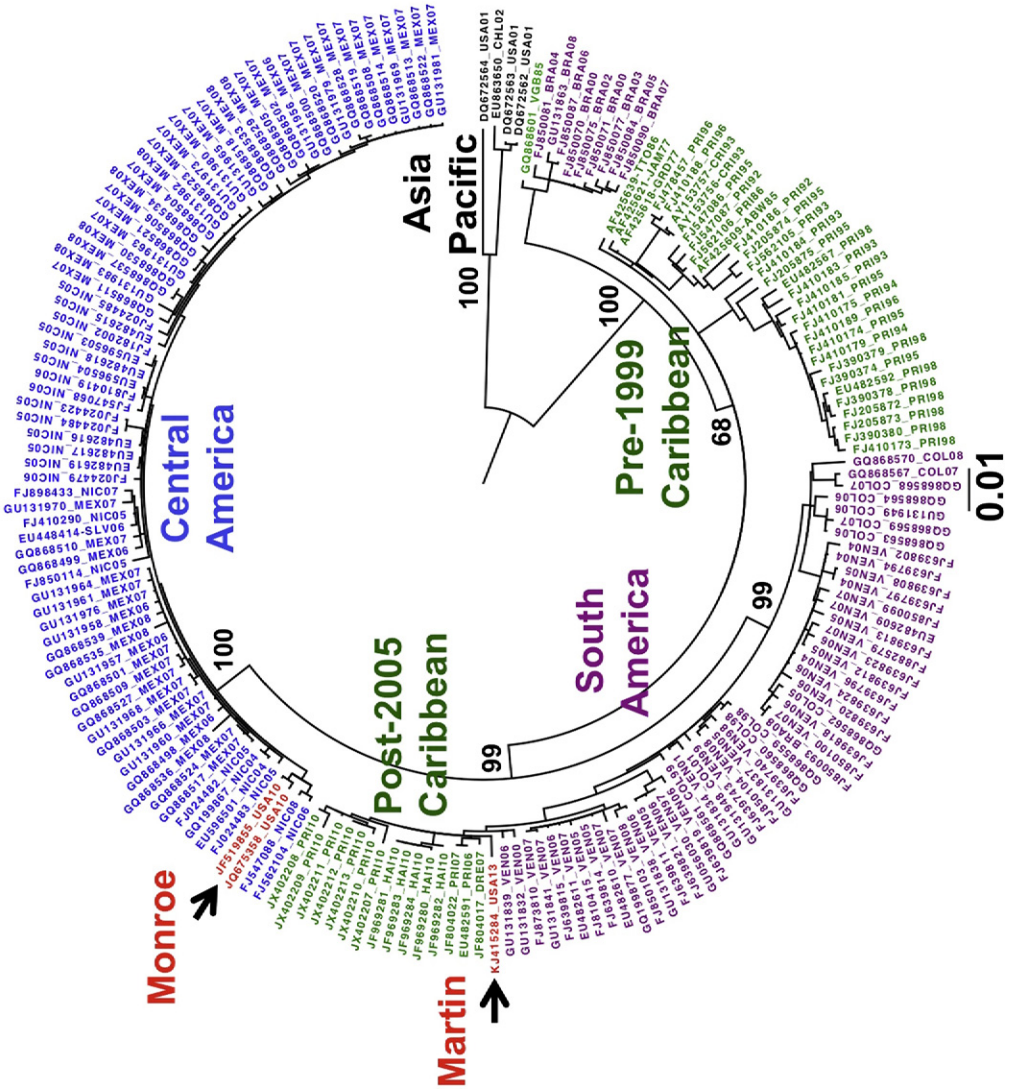


Fig. 1. Map of Florida showing counties with reported locally transmitted DENV cases in 2009–2013. Old Town Key West (open triangle) in Monroe County, the site of the 2009–2010 DENV-1 outbreak, is approximately 275 miles from the sites of the 2013 DENV-1 outbreak, Jensen Beach and Rio (open circle) in Martin County. Data were derived from the Florida Department of Health Mosquito-borne Diseases Surveillance reports (http://www.floridahealth.gov/diseases-and-conditions/mosquito-borne-diseases/surveillance.html).

2. Results

Semi-nested reverse transcriptase PCR (RT-PCR) was used to identify viremic samples and distinguish the infecting serotype in 13 serum samples collected between August 2 and September 26, 2013 from patients with dengue symptoms and no travel history in Martin County, Florida (Lanciotti et al., 1992). A single patient sample from August 29, 2013 was positive for DENV by first round RT-PCR. No other RT-PCR reaction from any of the other 12 patient samples showed a product consistent with amplification of DENV sequences. The second round, semi-nested PCR from the positive sample identified the serotype as DENV-1. Additional RT-PCR was performed to amplify the complete E protein coding region and adjacent regions. The sequence of this amplification product has been deposited in GenBank (MartinFL_USA2013, accession number KJ415284). The Martin County DENV-1 E sequence was aligned with a set of 188 additional non-redundant American DENV-1 E sequences from the National Center for Biotechnology Information Virus Variation database (Resch et al., 2009). This set included the majority of deposited western hemisphere DENV-1 sequences, in addition to several isolates from Hawaii (USA) and Easter Island (Chile) that belong to Asian DENV-1 clades and served as an outgroup.

Maximum likelihood, maximum parsimony and distance methods using the 1480-nt E gene region yielded phylogenetic trees with very similar topologies. The maximum likelihood tree is shown in Fig. 2. Western hemisphere DENV-1 strains group in geographically bounded clades. Evidence for clade replacement was seen most strikingly with a group of recent sequences from the Caribbean that are


Asia Pacific
Pre-1999 Caribbean
South America
Post-2005 Caribbean
Central America
Monroe
Martin
100
100
68
99
99
0.01

related to earlier South American DENV-1 clades rather than Central American clades (Zhang et al., 2005). The clade containing Central American strains was separated from the clade containing South American and recent Caribbean strains with strong bootstrap support. The Key West 2010 strains grouped with Central American strains, as previously reported (Graham et al., 2011; Munoz-Jordan et al., 2013). The Martin County DENV-1 strain grouped most closely with viruses that have recently appeared in the Caribbean from South American origins. In total, 12 nucleotide changes were observed in the 1480 nt aligned E gene region between the Martin County DENV-1 and the most closely related Caribbean strain, a DENV-1 found in the Dominican Republic in 2007 (GenBank accession number JF804017). Comparison of the predicted translation products of this Dominican Republic strain and the Martin County strain showed 3 amino acid changes in E. Using DENV-1 E protein numbering, the three altered amino acids were valine at E protein position 55 changed to isoleucine in the Martin County sequence (V55I), threonine at position 160 changed to isoleucine (T160I) and lysine at position 394 changed to arginine (K394R).

3. Discussion

The 2010 Key West DENV-1 strain grouped most closely with Central American viruses originating in Nicaragua (Graham et al., 2011) whereas the 2013 Martin County DENV-1 strain originated from a clade of DENV-1 that recently appeared in the Caribbean from South American origins. These two viruses are clearly distinct and are not a result of a single DENV-1 introduction in Florida and subsequent local spread of the virus by humans and/or mosquitoes. The bootstrap values supporting the separation of the clades that distinguish these viruses are high, indicating strong statistical support.

While the Martin County DENV-1 strain groups most closely with Caribbean DENV-1 strains, the branch length between the Martin County DENV-1 strain and all of the related Caribbean DENV-1 strains is relatively long, indicating that the Martin County strain is not very closely related to any specific Caribbean strain that has been sequenced. One possible interpretation for this relatively long branch length is that the Martin County DENV-1 strain had been isolated for some time from its closest relatives, either in the Caribbean, or possibly in Florida. An alternate, and perhaps more likely, scenario is that recent surveillance and sequencing from the Caribbean islands are incomplete and the closest relatives of the Martin County DENV-1 virus have not been sampled to date. As a result, it is difficult to determine with certainty which Caribbean island may have been the source of the 2013 Martin County DENV-1 strain. The Martin County DENV-1 strain has three amino acid changes in the E protein compared to the closest relative from the Dominican Republic. Two of these changes (V55I and K394R) are relatively conservative and are also found in a number of other related Caribbean DENV-1 strains. The T160I change on the other hand, is non-conservative and is not found in any other related DENV-1 strain. This change is structurally located on the exposed surface of the E protein domain I near the domain I/II hinge region, and may play a role in viral entry or immune escape.

There are many similarities between Old Town, Key West and the Jensen Beach and Rio neighborhoods where DENV cases were reported in Martin County in 2013. One that stands out is that neither location is a major port of entry for either aviation or shipping. The number of locally acquired cases was large compared to other Florida counties, indicating that factors existed in both Key West and Martin County that favored local transmission that may not have existed in other sites of local transmission. Locally acquired DENV has been reported in recent years in a number of metropolitan areas in Florida, but transmission was limited to a few cases at most. Therefore, a port of entry with large traffic from DENV endemic countries (for example, Miami-Dade County) may not be sufficient to initiate local DENV

Fig. 2. Maximum-likelihood phylogenetic tree of the 1480-nt envelope gene region from western hemisphere DENV-1 isolates. Sequences are labeled with GenBank accession numbers, country, and year. Countries are given as standard 3-letter codes. Scale bar indicates the number of substitutions per site. The Martin County and Key West sequences are shown in red; Central American sequences, in blue; Caribbean sequences, in green; South American sequences, in purple, Asian/Pacific sequences, in black. Isolates from Hawaii (USA) and Easter Island (CHI) are of Asian origin and form an outgroup. Viruses group by geography and by year. The bootstrap values of the important nodes separating Central American from South American and recent Caribbean strains are shown. The tree was drawn by using FigTree software (http://tree.bio.ed.ac.uk/software/figtree).

transmission and spread. Additional factors need to be considered. As in Key West, many households and businesses in Jensen Beach and Rio maintain their windows open more than 50% of the time, have vegetation in more than 50% of their property, or keep open-air containers such as birdbaths or other receptacles (Radke et al., 2012 and G. Lemire, presented at the 85th Annual Meeting of the Florida Mosquito Control Association, Cape Coral, FL, 17 to 20 November 2013). These factors could contribute to increased human exposure to mosquito bites.

The study of DENV introduction and local transmission in Florida can inform dengue control efforts and epidemiological surveillance. In this instance, we were only able to obtain sequence data from an individual patient sample and it is possible that other strains were also involved. However, to date, there is no evidence to support spread of DENV from one county to another within the state of Florida. The 2009–2010 Key West DENV-1 strain has not resurfaced in Martin County or appeared elsewhere in Florida. The end of the Key West outbreak could be attributed to effective vector control, climatic conditions, or human behaviors that limited exposure to mosquito bites. It remains to be seen whether DENV transmission will resume next rainy season in Martin County.

Frequent travel to dengue endemic countries, transport of goods, the presence of mosquito vectors, and behaviors that promote exposure to mosquito bites contribute to new introductions of DENV in the US. Consistent with the northernmost range of *A. aegypti* and *A. albopictus*, Suffolk County, New York was the site of a locally acquired case of DENV in 2013 (Rochlin et al., 2013). Thus, recent sporadic outbreaks of local DENV transmission in the continental US are not limited to the Texas–Mexico border as they had been in the past. Public awareness about DENV and its symptoms will increase the likelihood that an infected traveler will seek medical care. Effective surveillance of imported dengue cases including rapid diagnosis of infected travelers, quarantine measures (avoidance of mosquitoes), and increasing vector control measures in the immediate vicinity of index cases will play key roles in preventing the spread of DENV in currently non-endemic regions. The key to coordinating and mobilizing these efforts will be effective communication between diagnosing physicians, state health departments, mosquito control districts, local government officials, university research laboratories, and the public at large.

4. Materials and methods

4.1. Source of blood samples

Patient blood samples were collected for diagnostic purposes. The use of de-identified, unused patient samples for DENV analysis was approved by the Institutional Review Boards of Martin Health System and Florida Gulf Coast University (protocol number: 2013-61).

Viral RNA was extracted from serum samples using a Qiagen RNeasy mini kit (Valencia, CA, USA) following the manufacturer's instructions. DENV RNA was amplified using pan-dengue virus consensus primers by RT-PCR with a Qiagen One-step kit (Qiagen, Valencia, CA, USA) (Lanciotti et al., 1992). A second semi-nested round of PCR was performed with serotype-specific primers using Invitrogen Platinum PCR Supermix High Fidelity (Invitrogen, Carlsbad, CA, USA) (Lanciotti et al., 1992). The pan-DENV and DENV serotype specific amplification products were visualized by agarose gel electrophoresis. For phylogenetic analysis, a larger, 2594 bp fragment containing the entire E gene was amplified by RT-PCR using the following primers:

Forward 1KB-3F 5′-GCACATGCCATAGGAACATCCATCAC-3′
Reverse 3KBR 5′-ACTTCTCCTGACCCTGCAGAGACCAT-3′.

Three separate RT-PCR amplifications were performed and the products were each directly sequenced using terminal and internal primers by Sanger sequencing at double or triple coverage (Functional Biosciences, Madison, WI, USA). Sequence run data were assembled and edited using DNASTAR LaserGene software. A total of 188 American DENV-1 sequences from 1980 to 2013 were obtained through NCBI Virus Variation database to build a dataset for phylogenetic analysis (http://www.ncbi.nlm.nih.gov/genomes/VirusVariation/). Sequences were aligned using the MUSCLE algorithm and phylogenetic trees were generated using the SeaView software package (Gouy et al., 2010).

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